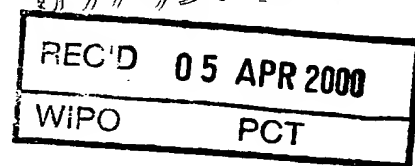




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I, KAY WARD, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PP 9414 for a patent by JAMES COOK UNIVERSITY filed on 23 March 1999.

WITNESS my hand this
Twenty-ninth day of March 2000

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PROVISIONAL SPECIFICATION

FOR THE INVENTION ENTITLED:

"ORGAN ARREST, PROTECTION AND PRESERVATION"

Applicant:

JAMES COOK UNIVERSITY

IP Australia
Documents received on:

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The invention is described in the following statement:

ORGAN ARREST, PROTECTION AND PRESERVATION

The present invention relates to a method and pharmaceutical or veterinary composition for arresting, protecting and/or preserving organs, in particular the heart during open-heart surgery.

The heart may be arrested for up to 3 hours during open-heart surgery. High potassium cardioplegia (in excess of 15-20 mM) has been the basis of myocardial arrest and protection for over 40 years. Currently the majority of solutions used contain high potassium including the widely used St Thomas No. 2 Hospital Solution which generally contains 110 mM NaCl, 16 mM KCl, 16 mM MgCl₂, 1.2 mM CaCl₂ and 10 mM NaHCO₃. Notwithstanding hyperkalemic solutions providing acceptable clinical outcomes, recent evidence suggests that progressive potassium induced depolarisation leads to ionic and metabolic imbalances that may be linked to myocardial stunning, ventricular arrhythmias, ischaemic injury, cell swelling and loss of function during the reperfusion period. The major ion imbalances are postulated to be due to an increased sodium influx which in turn activates the Na⁺/Ca²⁺ exchangers leading to a rise in intracellular Ca²⁺. Compensatory activation of Na⁺ and Ca²⁺ ion pumps then occur, which activate anaerobic metabolism to replenish ATP with a concomitant increase in tissue lactate and fall in pH. Free radical generation and oxidative stress have also been implicated in potassium arrest and partially reversed by the administration of antioxidants. In some cases, high potassium induced ischaemia has been reported to have damaged smooth muscle and endothelial function.

In an attempt to minimise ischaemic damage during cardioplegic arrest, an increasing number of studies have employed potassium channel openers instead of high potassium. Cardioprotection using nicorandil, aprikalim or pinacidil is believed to be linked to the opening of the potassium channel which leads to a hyperpolarised state, a shortening of the action potential and decreasing Ca²⁺ influx into the cell. One shortfall however is that the heart takes the same time or longer to recover than with high potassium cardioplegic solutions. Another is that pinacidil requires a carrier because of problems with solubility. The carrier

routinely used is dimethyl sulphoxide (DMSO) which is controversial when used in animal or human therapy.

Most investigators including those using potassium channel openers, believe that as soon as blood flow is halted, and the arrest solution administered, ischaemia occurs and progressively increases with time. In contrast, we sought a cardioplegic solution that would place the heart in a reversible hypometabolic state analogous to the tissues of a hibernating turtle, a hummingbird in torpor, an aestivating desert frog and a hibernating bear. When these animals drop their metabolic rate (some by over 90%), their tissues do not become progressively ischaemic but remain in a steady state. An ideal cardioplegic solution should produce a readily reversible, rapid electrochemical arrest without prolonged ischaemia. The heart should produce little tissue lactate, utilise little glycogen, show minimal changes in high-energy phosphates, cytosolic redox (NAD/NADH) and the bioenergetic phosphorylation (ATP/ADP Pi) ratio. There should be little or no change in cytosolic pH or free magnesium, minimal water shifts between the intracellular and extracellular phases, and no major ultrastructural damage to organelles such as the mitochondria. The ideal cardioplegic solution should produce 100% functional recovery with no ventricular arrhythmia, cytosolic calcium overload or other pump abnormalities. There is no cardioplegic solution currently available which fulfils all these requirements. We have now found that the heart can be better protected during arrest and recovery by using the potassium channel opener adenosine and the local anaesthetic lignocaine.

The action of adenosine is controversial. Adenosine has been shown to increase coronary blood flow, hyperpolarise the cell membrane and act as a preconditioning agent via the ATP-sensitive potassium channel and adenosine related pathways including adenosine receptors notably the A1 receptor. Adenosine is also known to improve myocardial recovery as an adjunct to high potassium cardioplegia. Furthermore, adenosine can be used as a pretreatment (whether or not it is present in the arresting solution) to reduce lethal injury. In one study, adenosine was shown to rival potassium arrest solutions and more recently in blood

cardioplegia, it prevented post-ischaemic dysfunction in ischaemically injured hearts. Adenosine is usually added as an adjunct to potassium cardioplegia.

Lignocaine is a known local anaesthetic which blocks sodium fast channels and has antiarrhythmic properties by reducing the magnitude of inward sodium current. The accompanying shortening of the action potential is thought to directly reduce calcium entry into the cell via Ca^{2+} selective channels and $\text{Na}^+/\text{Ca}^{2+}$ exchange. Recent reports also implicate lignocaine with the scavenging of free radicals such as hydroxyl and singlet oxygen in the heart during reperfusion. Associated with this scavenging function, lignocaine may also inhibit phospholipase activity and minimise membrane degradation during ischaemia. Lignocaine has also been shown to have a myocardial protective effect and in one study was found to be superior to high potassium solutions. However, our experiments show that lignocaine alone at 0.5, 1.0 and 1.5 mM gave highly variable functional recoveries.

According to one aspect of the present invention there is provided a method for arresting, protecting and/or preserving an organ which includes administering effective amounts of (i) a potassium channel opener or agonist and/or an adenosine receptor agonist and (ii) a local anaesthetic to a subject in need thereof.

According to another aspect of the present invention there is provided the use of (i) a potassium channel opener or agonist and/or an adenosine receptor agonist and (ii) a local anaesthetic in the manufacture of a medicament for arresting, protecting and/or preserving an organ.

The present invention also provides (i) a potassium channel opener or agonist and/or an adenosine receptor agonist and (ii) a local anaesthetic for use in arresting, protecting and/or preserving an organ.

According to a further aspect of the present invention there is provided a pharmaceutical or veterinary composition which includes effective amounts of (i) a potassium channel opener or agonist and/or an adenosine receptor agonist and (ii) a local anaesthetic.

While the present invention is particularly advantageous in arresting, protecting and/or preserving an organ while it is intact in the body of the subject, it

will be appreciated that it may also be used to arrest, protect and/or preserve isolated organs.

Thus, the present invention still further provides a method for arresting, protecting and/or preserving an organ which includes adding a composition which includes effective amounts of (i) a potassium channel opener or agonist and/or an adenosine receptor agonist and (ii) a local anaesthetic to the organ.

The term "adding" is used herein in its broadest sense to refer to any methods of exposing the organ to the composition of the present invention, for example, bathing, perfusing or pumping via various routes.

The term "organ" is used herein in its broadest sense and refers to any part of the body exercising a specific function including tissues and cells. Examples include circulatory organs such as the heart, respiratory organs such as the lungs, urinary organs such as the kidneys or bladder, digestive organs such as the stomach, liver, pancreas or spleen, reproductive organs such as the scrotum, testis, ovaries or uterus and neurological organs such as the brain. The method of the present invention is particularly useful in arresting, protecting and/or preserving the heart during open-heart surgery.

Thus, the present invention also provides a cardioplegic or cardioprotectant composition which includes effective amounts of (i) a potassium channel opener or agonist and/or an adenosine receptor agonist and (ii) a local anaesthetic.

The potassium channel openers or agonists may be selected from nicorandil, diazoxide, minoxidil, pinicadil, aprikalim, cromokulim, NS-1619 (1,3-dihydro-1-[2-hydroxy5(trifluoromethyl)phenyl]5-(trifluoromethyl)2-H-benzimidazol-one), amlodipine, Bay K 8644(L-type)(1,4-dihydro-26-dimethyl-5-nitro-4[2(trifluoromethyl)phenyl]-3-pyridine carboxylic acid (methyl ester)), bepridil HCl (L-type), calcisepetine (L-type), omega-conotoxin GVIA (N-type), omega-conotoxin MVIIC (Q-type), cyproheptadine HCl, dantrolene sodium (Ca^{2+} release inhibitor), diltiazem HCl (L-type), flodipine, flunarizine HCl ($\text{Ca}^{2+}/\text{Na}^{+}$), fluspirilene (L-type), HA-1077 2HCl(1-(5 isoquinolinyl sulphonyl) homo piperazine.HCl), isradipine, loperamide HCl, manoalide (Ca^{2+} release inhibitor), nicardipine HCl (L-type), nifedipine (L-type), nifedipine HCl (L-type), nimodipine (L-type), nitrendipine (L-

type), pimozide (L- and T- type), ruthenium red, ryanodine (SR channels), taicatoxin, verapamil HCl (L-type), methoxy-verapamil HCl (L-type), YS-035 HCl (L-type)N[2(3,4-dimethoxyphenyl)ethyl]-3,4-dimethoxy N-methyl benzene ethaneamine HCl) and AV blockers such as verapamil and adenosine. It will be appreciated that this list includes calcium antagonists as potassium channel openers are indirect calcium antagonists.

Adenosine is particularly preferred as it is capable of opening the potassium channel, hyperpolarising the cell, depressing metabolic function, possibly protecting endothelial cells, enhancing preconditioning of tissue and protecting from ischaemia or damage. Adenosine is also an indirect calcium antagonist, vasodilator, antiarrhythmic, antiadrenergic, free radical scavenger, arresting agent, anti-inflammatory agent (attenuates neutrophil activation), metabolic agent and possible nitric oxide donor.

Suitable adenosine receptor agonists include N⁶-cyclopentyladenosine (CPA), N-ethylcarboxamido adenosine (NECA), 2-[p-(2-carboxyethyl)phenethyl-amino-5'-N-ethylcarboxamido adenosine (CGS-21680), 2-chloroadenosine, N⁶-[2-(3,5-dimethoxyphenyl)-2-(2-methoxyphenyl)ethyladenosine, 2-chloro-N⁶-cyclopentyladenosine (CCPA), N-(4-aminobenzyl)-9-[5-(methylcarbonyl)-beta-D-robofuranosyl]-adenine (AB-MECA), ([IS-[1a,2b,3b,4a(S*)]]-4-[7-[[2-(3-chloro-2-thienyl)-1-methyl-propyl]amino]-3H-imidazole[4,5-b]pyridyl-3-yl]cyclopentane carboxamide (AMP579), N⁶-(R)-phenylisopropyladenosine (R-PLA), aminophenylethyladenosine 9APNEA) and cyclohexyladenosine (CHA).

The local anaesthetic can be selected from mexiletine, diphenylhydantoin or Class 1B antiarrhythmic agents such as lignocaine or derivatives thereof, for example, QX-314. Lignocaine is preferred as it is capable of acting as a local anaesthetic probably by blocking calcium fast channels, depressing metabolic function, lowering free cytosolic calcium, protecting against enzyme release from cells, possibly protecting endothelial cells and protecting against myofilament damage. Lignocaine is also a free radical scavenger and an antiarrhythmic.

Thus, in a particularly preferred embodiment there is provided a method for

arresting, protecting and/or preserving an organ which includes administering effective amounts of adenosine and lignocaine to a subject in need thereof.

In another preferred embodiment there is provided a pharmaceutical or veterinary composition which includes effective amounts of adenosine and
5 lignocaine.

For ease of reference, the "potassium channel opener or agonist" and the "local anaesthetic" will hereinafter be referred to as the "active ingredients".

The method of the present invention involves the administration of effective amounts of the active ingredients for a time and under conditions sufficient for the
10 organ to be arrested, protected and/or preserved. The active ingredients may be administered separately, sequentially or simultaneously and in a single dose or series of doses.

The subject may be a human or an animal such as a livestock animal (e.g. sheep, cow or horse), laboratory test animal (e.g. mouse, rabbit or guinea pig) or a
15 companion animal (e.g. dog or cat), particularly an animal of economic importance.

The active ingredients may be administered by any suitable route, including oral, implant, rectal, inhalation or insufflation (through the mouth or nose), topical (including buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intrasternal and intradermal). Preferably, administration
20 in open-heart surgery applications will be achieved by mixing the active ingredients with the blood of the subject or subjects having a similar blood type. The active ingredients then enter the coronary circulation. However, it will be appreciated that the preferred route will vary with the condition and age of the subject and the chosen active ingredients.

25 While it is possible for one or both of the active ingredients to be administered alone, it is preferable to administer one or both of them together with one or more pharmaceutically acceptable carriers, diluents adjuvants and/or excipients. Each carrier, diluent, adjuvant and/or excipient must be pharmaceutically "acceptable" in the sense of being compatible with the other
30 ingredients of the composition and not injurious to the subject. The compositions may conveniently be presented in unit dosage form and may be prepared by

methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers, diluents, adjuvants and/or excipients.

The present invention also extends to a pharmaceutical or veterinary composition which includes the active ingredients and a pharmaceutically or veterinarily acceptable carrier, diluent, adjuvant and/or excipient.

Liquid preparations for administration prior to arresting, protecting and/or preserving the organ may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g. sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g. lecithin or acacia); non-aqueous vehicles (e.g. almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); preservatives (e.g. methyl or propyl-p-hydroxybenzoates or sorbic acid); and energy sources (e.g. carbohydrates such as glucose, fats such as palmitate or amino acid).

Compositions suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the composition isotonic with the blood of the intended subject; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The compositions may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

A preferred pharmaceutically acceptable carrier is a buffer having a pH of about 6 to about 9, preferably about 7, more preferably about 7.4. Suitable buffers include Krebs-Henseleit which generally contains 10mM glucose, 117 mM NaCl,

5.9 mM KCl, 25 mM NaHCO₃, 1.2 mM NaH₂PO₄, 1.12 mM CaCl₂ (free Ca²⁺=1.07mM), 0.512 mM MgCl₂ (free Mg²⁺=0.5mM) and 1.2mM P, St. Thomas No. 2 solution, Tyrodes solution which generally contains 10mM glucose, 126 mM NaCl, 5.4 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 0.33 mM NaH₂PO₄ and 10 mM HEPES (N-[2-hydroxyethyl]piperazine-N'-[2-ethane sulphonic acid] Fremes solution, Hartmanns solution which generally contains 129 NaCl, 5 mM KCl, 2 mM CaCl₂ and 29 mM lactate and Ringers-Lactate.

In a further preferred embodiment, the present invention provides a pharmaceutical or veterinary composition which includes adenosine, lignocaine and Krebs-Henseleit buffer.

The composition may also advantageously be presented in the form of a kit in which the active ingredients are held separately for separate, sequential or simultaneous administration.

The invention will now be described with reference to the following example. This example is not to be construed as limiting in any way.

In the example, reference will be made to the accompanying drawing in which:

Figure 1, is a graph of aortic flow vs time comparing hearts arrested using 100µM adenosine and 0.5 mM lignocaine in Krebs-Henseleit and St. Thomas Hospital No. 2 solution.

EXAMPLE

This example compares the effects of adenosine (100µM) cardioplegia with hyperkalemic St. Thomas Hospital No. 2 solution (16 mM K⁺) on functional recovery after a period of global ischaemia.

Hearts from male 450g Sprague-Dawley rats (n=19) were perfused for 30 minutes in the working mode (preload 7.5 mmHg; afterload 100 mmHg) with Krebs-Henseleit pH 7.4 buffer at 37°C. Hearts were then arrested in a retrograde mode at a constant pressure of 70 mmHg with either (i) a solution containing 100 µM adenosine and 0.5 mM lignocaine in filtered Krebs-Henseleit (10 mM glucose, pH 7.6 – 7.8 @ 37°C) (n=11) or (ii) St. Thomas No 2 solution (0.2 micron filter) (n=8). Following 30 minutes of arrest, the hearts were switched back to normal

antegrade perfusion with Krebs-Henseleit pH 7.4 @ 37°C. Heart rate, coronary flow, aortic flow, aortic pressure and oxygen consumption were monitored every 10 minutes for 30 minutes. Statistical significance was assessed using a Student t-Test.

Results

- 5 Hearts arrested using adenosine cardioplegia achieved quiescence in half the time compared to St. Thomas No. 2 solution (30 vs 75 seconds, $p < 0.0001$). During arrest under a constant perfusion pressure, coronary blood flow was 30% greater using adenosine cardioplegia ($p < 0.05$). Adenosine arrested hearts also recovered faster (89 ± 10 vs 119 ± 30 seconds), but because of the greater variability in St.
- 10 Thomas group the difference was not significant. Recovery of all functional parameters at 5 minutes was significantly better in the adenosine group ($p < 0.05$); aortic flow (24 vs 9 ml/min), coronary flow (14 vs 9 ml/min), O_2 consumption (5.0 vs $2.3 \mu\text{mol/min/g wet wt}$) and heart rate (213 vs 113 bpm). Over the next 30 minutes all measured functional parameters in each group were not significantly
- 15 different. The results are summarised in Table 1 below and shown in Figure 1.

Table 1.

	Adenosine and Lignocaine	St. Thomas No. 2 Solution
Time to electrical and mechanical arrest	30 sec (n=11)	75 sec (n=8) ($p < 0.0001$)
5 Minute recovery		
Heart rate (bpm)	213	113 ($p < 0.05$)
Aortic flow (ml/min)	24	9.1 ($p < 0.05$)
Coronary flow (ml/min)	14	9.2 ($p < 0.05$)
MVO ₂ ($\mu\text{mol/min/g wet wt}$)	5.0	2.3 ($p < 0.05$)

- In terms of functional parameters, 100 μM adenosine and 0.5 mM lignocaine cardioplegia lead to shorter arrest times and an enhanced recovery profile compared
- 20 to the St. Thomas Hospital No. 2 solution.

Since modifications within the spirit and scope of the invention may be readily effected by persons skilled in the art, it is to be understood that the invention

is not limited to the particular embodiment described, by way of example, hereinabove.

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